Remarks

The Office Action, dated August 12, 2002, has been carefully considered. The claims have been amended to more clearly set forth the Applicants' contributions to the art and do not introduce new matter into the disclosure of the invention. The basis for the amendments to the claims can be found on page 1, lines 24-25, page 2, lines 1-3, 22-25, page 5, lines 1-7, page 12, lines 15-19, page 14, lines 25-28, page 15, lines 1-10, page 16, lines 10-13, 18-29, page 17, lines 1-7, page 29, lines 3-5, and page 18, lines 5-7, 11-16 of the Specification. It is believed that no additional fee is required as the number of independent and dependent claims is the same as originally filed. The amendments are those that the Examiner has indicated would address the issues raised by the application.

Before responding to the individual comments by the Examiner, it is believed that it would be helpful to first review the main points of the present invention so that the critical elements can be understood and so that the claims may be amended to fully encompass the invention's contribution to the arts.

First, the present invention is an immunoassay for use in detecting and monitoring analyte production *in vivo*. Previously described methods use a binding molecule to bind the analyte and immediately measure the quantity of analyte present at a given time. The present methods provide for the injection of a targeting moiety to bind the analyte as it is produced so that after a set period of time, typically from about 1 hour to about 72 hours, a blood sample is taken from the subject for testing the amount of analyte secreted over a period of time (see page 15, lines 3-10). The present invention provides the capability of measuring basal as well as stimulated peptide or protein cytokine or hormones production.

Second, the present method preserves the analyte over a set period of time by preventing analyte destruction, utilization, and excretion so that analyte will accumulate in extracellular fluids, including blood. Because the labeled neutralizing targeting molecule binds the analyte causing the analyte to be slow clearing, this causes the analyte, which may normally have a very short *in vivo* half life, to accumulate *in vivo* as a targeting moiety:analyte conjugate. Therefore, in the present invention, cytokines and other secreted peptides can be accurately measured in the blood even in the presence of binding proteins, which mask the peptide's presence in conventional assays (see page 12, lines 15-19).

Specifically, the present invention provides a method of measuring the ***production*** of a target analyte (a secreted peptide or protein hormone) of interest in a human or animal, using the following steps:

a. a subject is injected with an amount of labeled neutralizing targeting moiety, which binds specifically to the target analyte, in sufficient quantity that a measurable fraction of secreted target analyte is bound by the labeled neutralizing targeting moiety - the labeled targeting moiety may be injected subcutaneously or intravenously (see page 29, lines 3-5);

b. in the circulation, the labeled neutralizing targeting molecule is brought in proximity to the target analyte, the targeting moiety binds the circulating target analyte and produces a targeting moiety:analyte conjugate so that at this point, the target analyte is attached to a slowly clearing targeting moiety (*e.g.*, antibody, antibody fragment, soluble analyte receptor, fusion protein, or other slowly clearing anti-ligand moiety), which decreases the clearing rate of the active target analyte (see page 14, lines 25-28, page 15, lines 1-2 and page 16, lines 10-13);

c. after a sufficient period of time to allow accumulation of the conjugates, typically from about 1 hour to about 72 hours, a blood sample is taken from the host for testing the amount of targeting moiety:analyte conjugates (see page 15, lines 3-10);

d. a capture moiety, which recognizes a site on the analyte that is distinct from the site bound by the injected targeting moiety, is used to bind the targeting moiety:analyte conjugates to form targeting moiety:target analyte:capture moiety complexes; and

e. the amount of labeled targeting moiety:target analyte conjugate bound to the capture moiety provides a measure of the production of secreted target analyte in the subject over the defined period of time.

It should be particularly noted that following injection and distribution of the labeled neutralizing targeting molecule, secreted target analyte will be bound by this molecule as the analyte is produced *in vivo* over time and will form a soluble complex, which will accumulate

in blood and in other sites to which the labeled neutralizing targeting molecule has distributed and that soluble complex produced at sites other than blood will flow into the blood via the lymph, so that blood concentrations of complex are affected by complexes produced at other sites in the organism. (see page 16, lines 24-29 and page 17, lines 1-7)

Prior methods have been unable to accurately measure analyte production, *e.g.*, for cytokines, because of rapid excretion, catabolism, and utilization of cytokines as well as the binding of cytokines to endogenous cytokine binding proteins, which can interfere with detection. The present invention obviates all of these difficulties. (see page 5, lines 1-7) The main advantages are that this technique allows for:

(1) The accumulation in serum of a secreted or shed biological material that normally has a short *in vivo* half-life. The use of a labeled antibody or other molecule that binds the biological material greatly facilitates the measurement of the biological material in serum. No other technique has been described that allows quantitation of the amount of a biological material that has been secreted over a fixed, definable period of time *in vivo*.

(2) Repeated measurements of analyte production over time can be made in individual humans or experimental animals; and

(3) Measurement of analyte production that is little influenced by the presence of endogenously produced soluble analyte receptors.

Previous methods were ineffectual because there is typically too little analyte present to measure in an instantaneous fashion. The present method takes a new approach to allow sufficient time for a sufficient amount of secretion of the analyte after injection to obviate this problem. In addition, it was previously believed in the art that one might not be able to get a targeting moiety to the required analyte or that to do so would require too much targeting moiety to be practical or that sufficiently large quantities would block the very processes being studied. Many peptides have serum binding proteins or receptors that would again require impractical amounts of targeting moiety but it was surprisingly discovered that the present methods work as found.

Rejecti ns Under 35 U.S.C. 112, First Paragraph

The Examiner has rejected claims 1, 4-23 and 25-42 under 35 U.S.C. 112, first paragraph as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor, at the time the application was filed, had possession of the claimed invention. Specifically, the Examiner contends that the specification does not provide any literal support for the recitation of "in excess of measurable quantities of target analyte" in step (a). Therefore, the Examiner contends that this limitation constitutes new matter.

The claims have now been amended to include a description of the limitation as "injecting the human or animal with an amount of labeled neutralizing targeting moiety, which binds specifically to the target analyte, in sufficient quantity that a measurable fraction of secreted target analyte is bound by the labeled neutralizing targeting moiety." Support for this amendment can be found on page16, lines 18-23 and further on page 18, lines 11-14.

The Examiner has further rejected claims 1, 4-23 and 25-42 under 35 U.S.C. 112, first paragraph contending that these claims, while being enabled for *in vivo* targeting, *in vivo* capturing, and measuring production of secreted cytokines in the blood, did not provide reasonable enablement for *in vivo* targeting, *in vitro* capturing, and measuring of any other secreted proteins in the peripheral blood. Applicants respectfully traversed this rejection. While the Examiner has given a very thorough and detailed description of the reasons that the Examiner believes the disclosure does not meet the enablement requirement, Applicants respectfully disagree with the Examiner that the amount of experimentation required to make and use the present invention is undue. However, the present claims have been amended to clarify that the method requires the injection of labeled neutralizing targeting moiety in sufficient quantity that a "*measurable fraction* of secreted target analyte is bound by the targeting moiety," as described in the specification on page 16, lines 19-24 and further on page 18, lines 13-16.

The Examiner has evaluated the claims based on the scope of the target analyte in light of the teachings within the specification. The Examiner must consider whether or not there is sufficient detail on measuring endogenous analyte production and if there is recognition in the art of a correlation between measuring endogenous cytokine production and measuring endogenously produced peptides and protein hormones secreted in a human or animal, to enable the breadth of the claimed invention. These factors are determined by what

is disclosed in terms of working examples in the specification, teachings of unpredictable parameters, what is known in the art, predictability of the art for the claim scope, correlation of working examples to the claimed invention, and correlation of working examples in the prior art to the claimed invention.

Applicants respectfully submit that the specification provides adequate direction to those skilled in the art and that the disclosure would not require undue experimentation. Furthermore, the claims have now been amended to more clearly set forth the invention as a method of measuring the production of the secreted peptide or protein. As specifically described on page 18, lines 5-7, the methods of the present invention can be used to measure molecules other than cytokines, that are secreted, shed, or otherwise produced *in vivo*, including, but not limited to, hormones, peptides, and drug metabolism products. As fully described in section 6 of the Examiner's Office Action, this determination can be made based on the factors to be considered as follows:

The nature of the invention. Applicants submit that the very nature of the invention lends itself to the fact that the amount of experimentation required to perform the broadly claimed methods is not undue. This factor supports the Applicants' contention that the disclosure does not require undue experimentation since methods of binding to or capturing peptide or protein hormones, including cytokines, were well known in the art at the time of filing and the present invention utilizes a similar system. It was well known in the art at the time of filing the present invention that the macromolecule peptide and protein hormones will bind to the targeting moiety and capture moiety in a similar fashion. The Examiner has not fully considered the classification of cytokines and peptide hormones. All of the secreted peptides, including hormones, are physically similar and cannot be distinguished on the basis of any classification as a cytokine. As defined in "Fundamental Immunology," known in the art as the definitive textbook of immunology, "Cytokines are proteins that are secreted by cells and exert actions on either the cytokine-producing cell (autocrine actions) or on other target cells (paracrine actions)." [Leonard, Warren J., *Type I cytokines and interferons and their receptors, in Fundamental Immunology*, 4th Edition, Edited by William E. Paul, Lippincott-Raven Publishers, Philadelphia, 1999, p. 741.]

Therefore, a cytokine is any peptide that has been found to have an effect on the immune system. It is not classified as such on the basis of any physical property but merely

denotes an effect on the immune system by certain peptides. The fact that a certain peptide does or does not have an effect on the immune system will not have any bearing on the measurement of such a peptide by the present methods. Prolactin, although classified as a peptide hormone by endocrinologists, has an effect on the immune system and, therefore, may be deemed a "cytokine" by immunologists. There is no reason to believe that one secreted peptide would behave differently from another in the context of the present invention. Therefore, the present application provides a sufficient representation of the entire class of secreted peptides in its description.

The predictability of the art and the state of the prior art. The Examiner appears to have taken the stance that there is no predictability of the methods if the instant specification does not specifically describe the examples of all secreted peptides and protein hormones that are produced *in vivo*. The Examiner has merely made a bald statement that "there is no predictability based on the instant specification that the claimed method will work in any and all secreted peptide or protein hormones that are produced *in vivo* in humans and animals." The Examiner fails to appreciate that the present invention merely makes use of a well-established principle that various paratopic or binding molecules may bind peptides and protein hormones. The Examiner has provided no evidence that other peptides or protein hormones would not act predictably in the present methods.

Applicants respectfully traverse this rejection in light of the showing of working examples in the specification, the predictability of the art for the claim scope, the correlation of working examples of the claimed invention, and the correlation of working examples in the prior art to the claimed invention.

Working examples and guidance in the specification. The Examiner contends that in order to overcome the unpredictability of using other peptides or protein hormones in the present method, the specification would have to show examples of the instant invention overcoming the recognized obstacles. The Examiner seems to indicate that it is necessary to show working examples comparing a cytokine to the use of peptides or protein hormones. Cytokines are peptides that would behave similarly to other peptides or protein hormones. Applicants have provided sufficient detailed examples in the specification showing the use of cytokines, one of the largest groups of secreted peptides in a mammal. While the Examiner contends that "the specification does not establish a direct correlation between cytokines and

other peptides or protein hormones," those skilled in the art will have clear guidance in the prior art that all peptides have sufficient structural similarities to behave in a similar functional manner using the methods of the present invention.

The Examiner has not provided convincing evidence as to why she believes that the results achieved using the art-recognized cytokine model disclosed in Applicants' specification *cannot* be correlated to efficacy with other secreted peptides or protein hormones.

Quantity of experimentation and relative skill in the art. The Examiner contends that it would require an undue amount of experimentation for the skilled artisan to making use of the method as claimed. However, the Examiner has offered no evidence on this point other than a bald assertion. While it is true that the present invention would require some experimentation to determine the proper peptide or protein hormone and targeting moiety combinations required, *etc.*, the level of experimentation required is not high relative to the level of skill in the art and complexity of the art. Applicants have provided adequate working examples that show the use of different cytokines, as a class of peptide and protein hormones, will work in the present invention. The determination of the appropriate binding partners and dosages of targeting and capture moieties effective in measuring secretion would necessarily be determined empirically and would be merely routine. Dosages of binding agents are routinely extrapolated by methods known in the art.

The quantity of experimentation needed to be performed by one skilled in the art is only one factor involved in determining whether undue experimentation is required to make and use the invention. An extended period of experimentation may not be undue if the skilled artisan is given sufficient direction or guidance and sufficient guidance is available within the specification and prior art to readily perform the present methods. "The test is not merely quantitative since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed." *In re Wands*, 8 USPQ2d at 1404 (CCPA 1076). The time and expense are merely factors in this consideration and are not the controlling factors.

Any person skilled in the present art may easily ascertain the secreted peptides and protein hormones and their corresponding targeting and capture moieties that fall within the

scope of the present claims. The scope of the claims requires that an appropriate binding partner bind and neutralize the secreted peptide or protein hormone, which then require an appropriate capture moiety as well known in the art. Therefore, secreted peptides or protein hormones that do not meet these criteria will inherently fall outside the scope of the present claims.

The breadth of the claims. Although the Examiner contends that the breadth of the claims is very broad in that the instant claims are directed to a method for measuring *in vivo* production of secreted peptides or protein hormones as a target analyte of interest, the claim is not overly broad because Applicants have disclosed the concise mechanism of action that the claims of the present application require -- *i.e.*, that it requires binding of the target moiety to the peptide or protein hormone forming complexes between them *in vivo* and then reacting the complexes in vitro with a capture moiety for detecting and measuring the amount of peptide or protein complexes in assay detection method -- and that mechanism defines the breadth of the materials that work. Therefore, the claims are not as broad as indicated by the Examiner. They cover the true scope of the present invention and embodiments falling within those claims are easily determined by those skilled in the art by commonly known procedures.

It is well within the scope of ability of one skilled in the art to test various secreted peptide and protein hormones and their corresponding targeting and capture moieties of the present invention for the following reasons: (1) the amount of testing required is relatively small especially since most of the work can be done with in vitro experiments as the proof of principle with the animal studies was already provided; (2) testing of any particular secreted peptides or protein hormones in question would not require direction or guidance beyond that known in the art; (3) the current state of knowledge in the art and relative skill of those in the art is quite high; (4) well-known procedures exist for testing secreted peptides or protein hormones capable of working in the present methods; and (5) determining whether or not a secreted peptide or protein hormone falls within the scope of the claims is quite straightforward since all of the materials and methods that would be required to determine if a particular secreted peptide or protein hormone forms a necessary targeting moiety:target analyte:capture moiety complex are well-known in the art.

Rejection Under 35 U.S.C. 103

The Examiner has rejected claims 1 - 23 and 25 - 42 under 35 U.S.C. 103(a) as being unpatentable over Tamarkin, *et al.* (U.S. 5,587,294) in view of Pouletty, *et al.* (U.S. 5,612,034). Applicants maintain the arguments of record and respectfully traverse this rejection.

The present invention as defined by the amended claim 1 relates to a method of measuring analyte production *in vivo* over the set period of time using a method of injecting a targeting moiety to bind to an analyte that preserves the analyte by preventing analyte destruction, utilization, and excretion so that analyte will accumulate in extracellular fluids, including blood. Because the targeting molecule slows the clearing of the analyte, the analyte accumulates *in vivo* as a targeting moiety:analyte conjugate in order to acquire a measurable quantity.

The Examiner contends that it would have been obvious to one of ordinary skill in the art to incorporate the teaching of Pouletty *et al.* in injecting specific targeting moiety's into peripheral blood circulation for binding specifically with endogenous target analytes with the method of Tamarkin *et al.* wherein a blood sample from the patient is obtained in order to measure the amount of endogenously produced cytokines using competitive solid phase immunoassays. Specifically, the Examiner contends that Tamarkin has shown the difficulty in obtaining an accurate measurement of endogenously produced cytokines because of a masking effect of binding proteins in the blood. The Examiner further contends that, given that knowledge, someone in the art would then have found it obvious to specifically target the secreted analyte *in vivo* with antibodies specific for the target analyte to yield an accurate amount and measure of the endogenous target analyte.

However, the Examiner is using hindsight in order to reach such conclusion now that the Applicants have provided all of the experimental details. The references cited by the Examiner do not provide the necessary specific motivation much less a reasonable expectation of the success in solving the issues of the present invention. Applicants have submitted declarations demonstrating that one of ordinary skill in the art of immunology and medical science would not have deduced the present invention upon reading the references cited by the Examiner either alone or in combination.

First, Tamarkin merely discloses a competitive solid phase immunoassay for measuring the concentration of proteins, as already well known in the art. Tamarkin fails to teach injecting a neutralizing targeting moiety in order to form a targeting analyte complex or that such a complex formed could increase the life span of the target analyte *in vivo* without interfering with any *in vivo* processes that depend on the macromolecule of interest. And specifically noted by the Examiner, prior methods have been unable to accurately measure cytokine production because of rapid excretion, catabolism, and utilization of cytokines as well as the binding of cytokines to endogenous cytokine binding proteins, which can interfere with detection. The present invention obviates all of these difficulties.

Second, Pouletty *et al.* teaches only that one can increase the *in vivo* biological half-life of a compound that normally has a short *in vivo* half-life by injecting it into an animal so that it binds covalently to a molecule that naturally has a long *in vivo* half-life. There is *no* suggestion within the Pouletty reference that the resulting bound target analyte could be sampled in any measurable fashion after binding but only that its survivability would be extended. Pouletty does not suggest that increasing the half-life would provide the results of the present invention by using an excess of a neutralizing binding molecule that prevents catabolism. There is no suggestion that this method could be used to measure an analyte production over time or even that such a method would work for measuring the amount of analyte at all. There is nothing at all in Pouletty or within the art that would suggest that a practical amount of analyte could be bound *in vivo* that could then be sampled and measured.

When combined, Tamarkin and Pouletty failed to teach that one might use a targeting moiety to bind the analyte and measure the production of that analyte secreted over a set period of time. As known in the art at the time the invention was made, one skilled in the art reading the Pouletty reference would have had no reason to believe that measurement of the analyte would have been possible given the likely problems posed by such a method.

It would not have been obvious from these references that the present method would work since there are so many things that could have gone wrong. First, the targeting moieties could have formed large complexes with analytes. Such complexes would be rapidly eliminated, defeating the goal of extending the in vivo half-life of the analyte. Second, the present methods could have required larger quantities of targeting moieties than would have been feasible to actually use in a subject animal. Third, it was also likely, as believed in the

art, that the cytokines were produced in such a directed (polarized) fashion that they would have bound their targets before any targeting moiety could have intercepted them for measurement. Fourth, it was possible that injected targeting moieties might be out-competed for analyte binding by endogenous analyte-binding molecules, such as soluble receptors. Finally, it would have been believed that the detection methods of the present invention might not have provided a detectable signal above background binding levels (a signal-to-noise problem). In fact, had one truly followed the direction of Pouletty, such a method could not have been used to measure the analyte production. The present Applicants were the first to discover the unexpected result that such a method could be devised to get a sampling of the peptide production rate.

Applicants submit that the present methods would not have been obvious in light of the cited references or any other references in the art as demonstrated by the fact that the present method has been well recognized throughout the field of immunology. As evidence, the Applicants submit that the methods of the present invention have been published in several professional peer reviewed journal articles and internationally recognized art-recognized text books and it is currently the subject of a royalty-bearing commercial license in the field.

If the methods of the present invention had been as obvious as the Examiner now contends, after the current applicants have provided a complete roadmap of the invention, then others would have put together the proper pieces and have come up with the same invention. However, despite such a long felt need in the art for such an important diagnostic method, no one else in the art had discovered that the present method would work.

To summarize the art cited by the Examiner, all that is disclosed in Tamarkin is that one may use an immunoassay to measure small molecules, *e.g.*, cytokines, and all that is disclosed in Pouletty is that one may increase the biological effectiveness of an analyte *in vivo* by binding ~~and~~ to a binding molecule. One skilled in the art would find nothing in Tamarkin and Pouletty, either alone or in combination that would teach or suggest the present invention or a motivation for making the present invention. Furthermore, there is no motivation to combine the references in such a way as to arrive at the claimed invention. These represent nothing more than an invitation to try to see whether any particular compounds could be useful in binding an analyte to try to make available for measurement.

At the very most, the Tamarkin and Pouletty references **might** suggest that its would have been "obvious to try" to use a targeting moiety in measuring the concentration of an analyte. As the Examiner well knows, "obvious to try" is not a valid basis for formulating a rejection under 35 U.S.C. 103. Even if one skilled in the art was tempted to see whether a particular analyte could be bound by a targeting moiety, the host of expected problems and difficulties would have led that person away from trying the current methods for measuring analyte production.

Therefore, the present invention is not obvious under 35 U.S.C. 103 and accordingly, an obviousness rejection under this section is improper and the Applicants respectfully request reconsideration and withdrawal of this rejection.

In view of the above, it is respectfully submitted that the claims as amended and presented before the Examiner are in condition for allowance. Accordingly, reconsideration and withdrawal of the rejections are requested and allowance of claims 1, 4-23 and 25-42 is earnestly solicited.

Respectfully submitted,

Fred D. Finkelman
Suzanne C. Morris

By _____
Stephen R. Albainy-Jenei
Registration No. 41,487
Attorney for Applicant(s)
FROST BROWN TODD LLC
2200 PNC Center
201 East Fifth Street
Cincinnati, Ohio 45202-4182
(513) 651-6839

CINlibrary/1232194.3

Serial No. 09/167,088

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS:

Please amend the following claims as indicated:

1. (Four times Amended) A method of measuring the production of a secreted target analyte of interest in a human or animal, comprising the steps of:

a. injecting the human or animal with an amount of labeled neutralizing targeting moiety, [capable of binding] wherein the targeting moiety binds specifically to the target analyte, and wherein the targeting moiety is injected in sufficient quantity that a measurable fraction of target analyte is bound by the labeled neutralizing targeting moiety [in excess of measurable quantities of target analyte];

b. allowing the targeting moiety to circulate through the injected human or animal for a defined period of time sufficient to bind to the target analyte of interest and form a targeting moiety:target analyte conjugate wherein the formation of the targeting moiety:target analyte conjugate decreases the clearing rate of the target analyte;

c. obtaining a sample of blood from the human or animal after the defined period of time;

d. combining the sample of blood with a capture moiety [capable of binding] wherein the capture moiety binds specifically to the targeting moiety:target analyte conjugate in order to form an assay mixture;

e. incubating the assay mixture of step d to allow the capture moiety to bind [specifically] to the targeting moiety:target analyte conjugate and form targeting moiety:target analyte:capture moiety complexes in the assay mixture;

f. removing any unbound and unconjugated targeting moiety and target analyte from the assay mixture;

g. detecting the amount of [bound] labeled targeting moiety:target analyte:capture moiety complexes [conjugate on the capture moiety];

h. wherein the amount of labeled targeting moiety:target analyte:capture moiety complexes detected in step (g) provides a measure of the production of secreted target

analyte in the sample during the defined period of time [determining the amount of the target analyte in the sample correlating to the amount of targeting moiety:target analyte conjugate bound to the capture moiety and detected in step (g)]; and

i. wherein the target analyte is a secreted cytokine or peptide or protein hormone or cytokine.

4. (Once Amended) The method of claim 14 [3], wherein the target analyte [protein] is a cytokine.

7. (Twice Amended) The method of claim 1, wherein the blood is selected from the group consisting of whole blood, serum and plasma.

13. (Thrice Amended) The method of claim 1, wherein the targeting moiety is labeled with a small molecule hapten and wherein the method further comprises the step of binding the small molecule hapten [by linking the targeting moiety to a label which label can then be bound] to a binding partner which is conjugated to an enzyme.

14. (Once Amended) The method of claim [13, wherein the label is a small molecule hapten] 1, wherein the defined period of time is from about 1 hour to about 72 hours.

15. (Once Amended) The method of claim 13 [14], wherein the hapten is biotin.

20. (Twice Amended) The method of claim 8, further comprising after step (a) the step of injecting the human or animal with an amount of second targeting moiety, wherein the second targeting moiety binds specifically to the first targeting moiety, wherein the second targeting moiety is injected in sufficient quantity that a measurable fraction of first targeting moiety is bound by the second targeting moiety and [wherein the targeting moiety is a first targeting moiety itself capable of being bound by a second targeting moiety] wherein the second targeting moiety is specifically bound [recognized] by the capture moiety.

25. (Once Amended) The method of claim 20, wherein the means for detecting the targeting moiety:target analyte:capture moiety complexes [bound conjugate on the solid support] is by radioimmunoassay[, wherein the molecule capable of binding the targeting moiety is labeled by linking the targeting moiety to a radioisotope].

Serial No. 09/167,088

26. (Twice Amended) The method of claim 20, wherein the second targeting moiety is detectably labeled by an enzymatic label [linking it to a label which label can then be bound to a binding partner which is conjugated to an enzyme].

31. (Once Amended) The method of claim 20, wherein the [molecule capable of binding the targeting] capture moiety is labeled by linking to a fluorescent labeling compound.

34. (Once Amended) A reagent kit useful in performing the method of claim 1, comprising

(a) a first reagent [containing] comprising a labeled targeting moiety specific for the target analyte wherein the label is an enzyme indicating means operatively linked to the targeting moiety [and capable of forming a conjugate with the target analyte];

(b) a second reagent separated from said first reagent, wherein the second reagent comprises [which contains] a capture moiety specific for the target analyte even when such target analyte is conjugated with the labeled targeting moiety; [for said conjugate] and

(c) a third reagent separated from said first and second reagents which contains a standard for the analyte.

37. (Twice Amended) A reagent kit useful in performing the method of claim 20 [1], comprising: (a) a first container having first targeting moieties comprising paratopic molecules that immunoreact with a target analyte, and are operatively linked to a label; (b) a second container having second targeting moieties comprising paratopic molecules that immunoreact with the target analyte at a site different from the first targeting moieties but are not in the first container; [and](c) a second reagent separated from said first reagent, wherein the second reagent comprises a capture moiety specific for the target analyte even when such target analyte is conjugated with the labeled targeting moiety; and (d) one or more other containers comprising one or more of the following: a sample reservoir, a solid phase support, wash reagents, reagents for [capable of] detecting the presence of the first targeting moieties [bound antibody] from the second container, or reagents for [capable of] amplifying the label.